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Community Reference Laboratory for Feed Additives

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CRL Evaluation Report on the Analytical Methods submitted in connection with the Application for Authorisation as a Feed Additive according to Regulation (EC) No 1831/2003

Dossier related to:	FAD-2009-0045 CRL/090029
Name of Additive:	Vitamin B6
Active Agent (s):	Pyridoxine Hydrochloride
Rapporteur Laboratory:	Laboratori Agroalimentari de Cabrils (LAC)
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EXECUTIVE SUMMARY

In the current application authorisation is sought for *Vitamin B6* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of *pyridoxine hydrochloride (Vitamin B6)* for all animal species and categories. The feed additive is an almost white crystalline powder with a minimum content of 99% of pyridoxine hydrochloride. Vitamin B6 is intended to be incorporated in feedingstuffs via *premixture.* Due to excellent solubility in water the additive can be used directly for water applications. The applicant does not propose any maximum or minimum concentration of feed additive in feed or water. Furthermore no limits are set in previous regulations.

For the determination of the active substance *pyridoxine hydrochloride (Vitamin B6)* in the *feed additive* the applicant proposes the titration method described in the European Pharmacopoeia (Ph.Eur.6th, monograph 0245). The CRL-FA considers this method suitable to be used within the frame of official control.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures* the applicant proposes a method of the Association of German Agricultural Analytical and Research Institutes (VDLUFA, Germany). The method is based on High Performance Liquid Chromatograpy (HPLC) coupled to an UV detector and was ring-trial validated on premixtures containing the target analyte at concentration from 627 to 11530 mg/kg. The following performance characteristics were reported:

- relative standard deviation for *repeatability* (RSD_r) ranging from 2.6 to 3.4% , and
- relative standard deviation for *reproducibility* (RSD_R) ranging from 4 to 5.1%.

Based on these acceptable performance characteristics the CRL considers this method suitable for the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures* within the concentration range covered by the collaborative study. Therefore the CRL-FA recommends this VDLUFA Bd.III, 13.9.1 method for official control to determine *pyridoxine hydrochloride (Vitamin B6)* in *premixtures*.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *feedingstuffs* and in *water,* the applicant did not submit any method. Therefore the CRL-FA cannot evaluate nor recommend any method for the determination of the active substance in these matrices.

No further testing or validation is required.

KEYWORDS

pyridoxine hydrochloride, vitamin B6, nutritional additive, vitamins, all animal species, all categories

1. BACKGROUND

In the current application authorisation is sought under articles 4(1) (new use in water) and 10(2) (re-evaluation of the already authorised additive under council directive 70/524/EEC) for *pyridoxine hydrochloride (Vitamin B6)* under the category/functional group 3(a) 'nutritional additives'/'vitamins, pro-vitamins and chemically well defined substances having similar effect' according to Annex I of Regulation (EC) No 1831/2003 [1]. According to the applicant the product is an almost white crystalline powder containing a minimum of 99% pyridoxine hydrochloride [2]. It is manufactured using 4-methyl-5-ethoxyl oxazole, n-propyl dioxepin and hydrochloric acid [3]. Authorisation is sought for the use of the feed additive for all animal species and categories [1]. The product is intended to be incorporated into compound *feedingstuffs* via *premixtures*, as direct dosage to mixed feed is not recommended. No minimum or maximum concentrations of the feed additive in feed or water are proposed by the applicant [2]. Furthermore no limits are set in previous regulation [4].

2. TERMS OF REFERENCE

In accordance with Article 5 of Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009, on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the duties and the tasks of the Community Reference Laboratory concerning applications for authorisations of feed additives, the CRL is requested to submit a full evaluation report to the European Food Safety Authority (EFSA) for each application. For this dossier, the methods of analysis submitted in connection with Vitamin B6, and their suitability to be used for official controls in the frame of the authorisation, were evaluated.

3. EVALUATION

Identification /Characterisation of the feed additive

Qualitative and quantitative composition of impurities in the additive

When required by EU legislation, analytical methods for official control of undesirable substances in the additive (e.g. arsenic, cadmium, lead, mercury, aflatoxin B1 and dioxins) are available from the respective Community Reference Laboratories [5].

Description of the analytical methods for the determination of the active substance in feed additive, premixtures, feedingstuffs and water

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in the *feed additive*, the applicant proposes the European Pharmacopoeia method (Ph.Eur. 6th Edition, monograph 0245) [6], based on a potentiometric titration with 0.1 M perchloric acid. In this assay 1 mL of 0.1 M perchloric acid is equivalent to 20.56 mg of $C_8H_{12}ClNO_3$. No performance characteristics of this method are provided. Nevertheless the CRL-FA recommends for official control the European Pharmacopoeia method to determine *pyridoxine hydrochloride (Vitamin B6)* in the *feed additive*.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures* the applicant proposes the VDLUFA Bd.III, 13.9.1 method of the Association of German Agricultural Analytical and Research Institutes (VDLUFA, Germany) [7]. The method allows for the simultaneous detection of vitamin B1, B2, B6, Nicotinic acid and Nicotinamide and is based on ion pair reversed phase (RP) High Performance Liquid Chromatograpy (HPLC) coupled to an UV detector measuring at 291 nm. The sample is extracted with a mixture of diethylenetriaminepentaacetic acid (titriplex V) and methanol and subjected without further clean-up to HPLC. The target analytes are quantified against external calibration.

The method was ring-trial validated on *premixtures* containing *pyridoxine hydrochloride (Vitamin B6)* at concentration ranging from 627 to 11530 mg/kg. The following performance characteristics were reported:

- relative standard deviation for *repeatability* (RSD_r) ranging from 2.6 to 3.4%, and
- relative standard deviation for *reproducibility* (RSD_R) ranging from 4 to 5.1%.

Based on these acceptable performance characteristics the CRL considers this method suitable for the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures* within the concentration range covered by the collaborative study. Therefore the CRL-FA recommends

the VDLUFA Bd.III, 13.9.1 method for official control to determine *pyridoxine hydrochloride (Vitamin B6)* in *premixtures*.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in the *feedingstuffs* the applicant did not submit any method. The VDLUFA method was not investigated for the *feedingstuffs*, due to potential matrix interference at the low vitamin concentrations, leading to high detection limits. Therefore the CRL cannot evaluate nor recommend this method for official control to determine *pyridoxine hydrochloride (Vitamin B6)* in *feedingstuffs*. A possible solution could be to use the abovementioned method using standard addition. However, this needs to be verified on a case by case basis.

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *water* the applicant did not submit any method. Due to the high solubility of the product in water, both European Pharmacopoeia and VDLUFA method could be applied to support this approach. However no experimental data were provided by the applicant for the aqueous media. Therefore the CRL cannot evaluate and therefore recommend these methods for official control to determine *pyridoxine hydrochloride (Vitamin B6)* in *water*.

No further testing or validation is required

4. CONCLUSIONS AND RECOMMENDATIONS

In the frame of this authorisation the CRL recommends for official control:

- the European Pharmacopoeia method (Ph. Eur. 6th edition, monograph 0245) using titration with perchloric acid for the determination of *pyridoxine hydrochloride (Vitamin B6)* in the *feed additive;*
- the VDLUFA Bd.III, 13.9.1 method, using ion pair reversed phase High Performance Liquid Chromatograpy coupled to UV detector (RP-HPLC-UV) for the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures*.

No methods are recommended by the CRL for official control to determine *pyridoxine hydrochloride (Vitamin B6)* in *feedingstuffs* and *water*.

Recommended text for the register entry (analytical method)

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in the *feed additive:*

- Titration with perchloric acid from European Pharmacopoeia (Ph. Eur. 6th edition, monograph 0245)

For the determination of *pyridoxine hydrochloride (Vitamin B6)* in *premixtures*:

- Reversed phase High Performance Liquid Chromatography coupled to UV detector (RP-HPLC-UV) - VDLUFA Bd.III, 13.9.1 method

5. DOCUMENTATION AND SAMPLES PROVIDED TO CRL

In accordance with the requirements of Regulation (EC) No 1831/2003, reference samples of Vitamin B6 have been sent to the Community Reference Laboratory for Feed Additives. The dossier has been made available to the CRL by EFSA.

6. REFERENCES

[1] *Application/Ref:SANCO/D/2:Forw. Appl. 1831/042-2009

[2] *Application, Proposal for Register Entry, Annex A

[3] *Technical dossier, Section II: Identity, characterisation and conditions of use of the additive

[4] COUNCIL DIRECTIVE 70/524/EEC of 23 November 1970 concerning additives in feeding-stuffs

[5] Commission Regulation (EC) No 776/2006 amending Annex VII to Regulation (EC) No 882/2004 of the European Parliament and of the Council as regards to Community Reference Laboratories

[6] *Technical Dossier, Section II, Annex 2.08 Method of Analysis: European Pharmacopoeia 6th Edition: Monograph 0245

[7] *Technical Dossier, Section II, Annex 2.09 HPLC VDLUFA Bd. III 13.9.1

* Refers to Dossier No. FAD-2009-0045

7. RAPPORTEUR LABORATORY & NATIONAL REFERENCE LABORATORIES

The Rapporteur Laboratory for this evaluation was Laboratori Agroalimentari de Cabrils (LAC), Departament d'Agricultura, Alimentació i Acció Rural de la Generalitat de Catalunya, Cabrils, Spain. This report is in accordance with the opinion of the consortium of National

Reference Laboratories as referred to in Article 6(2) of Commission Regulation (EC) No 378/2005, as last amended by Regulation (EC) No 885/2009.

8. ACKNOWLEDGEMENTS

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- Plantedirektoratet, Laboratorium for Foder og Gødning, Lyngby (DK)
- Schwerpunktlabor Futtermittel des Bayerischen Landesamtes für Gesundheit und Lebensmittelsicherheit (LGL), Oberschleißheim (DE)
- Landwirtschaftliche Untersuchungs- und Forschungsanstalt (LUFA) Speyer, Speyer (DE)
- Ústřední kontrolní a zkušební ústav zemědělský (ÚKZÚZ), Praha (CZ)
- Laboratoire de Rennes, SCL L35, Service Commun des Laboratoires, Rennes (FR)
- Univerza v Ljubljani, Veterinarska fakulteta. Nacionalni veterinarski inštitut, Enota za patologijo prehrane in higieno okolja, Ljubljana (SI)
- Sächsische Landesanstalt für Landwirtschaft, Fachbereich 8 — Landwirtschaftliches Untersuchungswesen, Leipzig (DE)
- Österreichische Agentur für Gesundheit und Ernährungssicherheit (AGES), Wien (AT)
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